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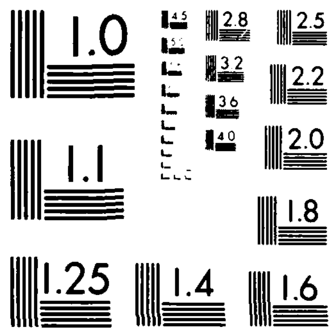

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KOREAN HEMORRHAGIC FEVER

(Hemorrhagic Fever with Renal Syndrome(HFRS))

Final Report

HO WANG LEE, M. D.

July 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-83-G-9526

Korea University College of Medicine
Seoul, Korea

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Hemorrhagic fever with renal syndrome (HFRS) was an important military problem since large epidemics of HFRS occurred among soldiers in the past wars. Although predominantly associated with field mice in rural areas, it is now being recognized that

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urban rats and laboratory rats are also reservoirs of Hantaan virus, the etiologic agent of HFRS, in many parts of the world.

This report presents the results of the isolation of Hantaan virus from blood of HFRS patients in tissue culture cells, the serosurvey of Hantaan virus among U.S. soldiers and wild rats caught at the U.S. Army Installations in Korea, and the serosurvey of domestic animals in Korea and neighbouring countries.

From blood of HFRS patients, 3 strains of Hantaan virus were isolated in Vero E-6 cells and 19 strains in Apodemus mice.

The prevalence rate of IF antibodies to Hantaan virus among 1,986 soldiers stationed in Korea was 1.2% which is a data very similar to that of Seoul residents. Of the 195 wild rats caught at the U.S. Army Installations, 10% had serum antibodies and viral antigen was found in lungs of 2 rats.

In domestic animals, IF antibodies to Hantaan virus were demonstrated in 3.5% of 792 commercial rabbits, 1 out of 123 chicken and 1 of 104 porcine sera. Originator [illegible]

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SUMMARY

Nineteen strains of Hantaan virus from blood of Hemorrhagic fever with renal syndrome (HFRS) patients were isolated in *Apodemus* mice and three strains of Hantaan virus in Vero E-6 cells. The presence of IF antibodies in certain blood specimens did not interfere with the virus isolation from those specimens.

Fatality rate of HFRS patient among U.S. soldiers hospitalized in the U.S. Army Hospital in Seoul is very high. The prevalence rate of IF antibodies to Hantaan virus among U.S. soldiers stationed in Korea was 1.2% which is a data very similar to that of Seoul residents. Urban rats captured at the U.S. Army Installation in Seoul were found to have IF antibodies to Hatnaan virus. Serum antibodies were detected in 12.3% of 141 *R. norvegicus* and in 7.4% of 54 *R. rattus*. Hantaan viral antigen was found in pulmonary tissues of 2 rats.

Commercial rabbits bought from breeding Co. in Korea and Japan were found to have IF antibodies to Hantaan virus. Serum antibodies were demonstrated in 3.5% of 792 New Zealand rabbits. One out of 123 chicken and 1 of 104 porcine sera were antibody positive for Hantaan virus. However, dogs, cattles, geese and ducks were negative for Hantaan virus.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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A. ISOLATION OF HANTAAN VIRUS FROM BLOOD SPECIMENS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME PATIENTS.

INTRODUCTION

During the Korean War, more than 2,400 United Nations troops stationed in the 38th Parallel in Korea developed a rare disease which had not been recognized previously by western physicians and this incident attracted great attention throughout the world (1). The illness became known as Korean hemorrhagic fever (KHF) and was accompained by an alarming mortality. Ten to fifteen percent of those affected died of shock and renal failure (2).

Hemorrhagic fevers with a syndrome very similar to KHF have been reported throughout Euro-Asia: (i) as hemorrhagic nephroso-nephritis in the Soviet Union, with several thousand cases annually since 1913 (3); (ii) as Songo fever or epidemic hemorrhagic fever (EHF) in China, with more than 32,000 cases reported in 1981 since 1931 (4,5); (iii) as nephropathia epidemica (NE) in Sandinavia, with several hundred cases reported anually since 1934 (6,7); (iv) as epidemic nephritis or epidemic hemorrhagic fever in Eastern Europe, since 1934 (8); and (v) as epidemic hemorrhagic fever in Japan, since 1960 (9).

This disease was an important military problem since large epidemics of hemorrhagic fever with renal syndrome(HFRS) occurred among soldiers in the past wars. More than 10,000 cases of EHF with 30% fatality occurred among Japanese soldiers in Manchuria during World War II (10). Several thousand cases of a War nephritis clinically similar to NE were reported among British soldiers stationed in Flanders during World War I (11,12). About 16,000 cases of NE among German troops in Laplands and in Yugoslavia during World War II (13) and about 14,000 cases of War nephritis which was very similar to NE were also described among Northern Armies of the Central Region during the American Civil War (12).

The causative agent of KHF was first discovered in 1976 from <u>Apodemus agrarius</u> mice (14), and then in 1978 the agent was isolated from KHF patients in Korea (15). The etiologic agent of KHF has been propagated in a human cell culture line (16), and it was named Hantaan virus after the Hantaan river which runs near the 38th parallel between South and North Korea (17).

A close etiological relationship was established among KHF in Korea, hemorrhagic nephroso-nephritis in USSR, NE in Scandinavia and EHF in Japan and in China (15,18,19,20).

At a WHO meeting in Tokyo, 22-24 Feb, 1982, the working group on HFRS recommended that the above-mentioned diseases with different names should be referred to as "Hemorrhagic fever with renal syndrome (HFRS)" (21).

Recent sero-epidemiologic surveys show that agents that are antigenically related to Hantaan virus are widely distributed

throughout much of the world to a much greater geographic extent than previously recognized. They are present not only in Euro-Asian Continents but also in American Continents and in Africa (22,23,24,25,26).

Analysis of the Hantaan virus genome (27,28) gave consistent finding with the morphological observations (29,30) which suggested that this virus be classified in the family Byunyaviridae. Recently, many viruses related by IF to Hantaan virus have been isolated from rodents both in endemic region and in many parts of the world with no known cases of HFRS (24,25,26,31). Until now, there has been no report on the isolation of Hantaan virus from blood specimens of HFRS patients by direct inoculation into tissue culture cells.

We now report here the successful isolation of Hantaan virus from the blood and serum specimens of HFRS patients in *Apodemus* mice and in Vero E6 cell cultures.

MATERIALS AND METHODS

Specimens from HFRS patients

As soon as HFRS patients were hospitalized, 10 to 20 ml of blood and urine were taken in heparinized tubes (B-D Co.,) and bottles, respectively, as initial samples for virus isolation. Some of these specimens were inoculated into *Apodemus* mice and Vero E-6 cells immediately while the remaining specimens were stored in -60°C until needed. More blood samples were taken from the patients at regular intervals during the course of their illness, and the sera from these samples to be used for antibody titration against Hantaan virus were stored in -60°C until needed.

Normal Apodemus agrarius

A. agrarius jejuensis mice were trapped in Jeju Island. The island had never documented cases of HFRS. Animals were free of Hantaan virus and antibodies in our preliminary studies and each animal serum were examined for antibodies to Hantaan virus before use. The mice weighted 20 - 40 g each.

Cell cultures

E-6 cells, a cloned line of Vero cells (CRL-1586), were obta[illegible] from Dr. J.M. Dalrymple, USAMRIID. E-6 cells were cultiv[illegible] in Eagle's minimal essential medium supplemented with 5[illegible] [illegible]l calf serum and 1% Hepes buffer.

Virus isol[illegible]ion

From [illegible]79 to 1982, 0.5 ml of blood and urine were inoculated into each *Apodemus* mice intramuscularly and subcutaneously using a 1 ml syringe and 26 gauge needle for virus isolation.

The animals were autopsied on the 20th day after inoculation. The virus was isolated from pulmonary tissue of *Apodemus* mice and ultimately identified as Hantaan virus by the neutralization

B. SEROEPIDEMIOLOGIC SURVEY OF HANTAAN VIRUS INFECTIONS AMONG U.S. ARMY SOLDIERS STATIONED IN KOREA AND IN WILD RATS CAPTURED AT THE U.S. ARMY INSTALLATIONS IN SEOUL.

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a tremendous amount of effort, time and money needed to spend for the study of this elusive new virus.

CONCLUSION

Hantaan virus was isolated using a reproducible technique from blood specimens of HFRS patients obtained during acute phase of illness, inoculated into *A. agrarius* mice and Vero E-6 cell cultures, and subsequently examined for identification of the virus by IF antibody technique and neutralization test.

19 strains of Hantaan virus from blood of HFRS patients were isolated in *Apodemus* mice and three strains of Hantaan viruses in Vero E-6 cells.

The presence of IF antibodies in certain blood specimens did not interfere with the viral isolation from those specimens.

A. agrarius coreae, and replicates in *A. agrarius coreae* without producing any signs of illness in the animals (15).

Until 1981, *Apodemus agrarius* mice was the only known detector system of Hantaan virus; therefore, *Apodemus* mice were used for isolation, propagation and neutralization of the virus. Finally, in 1981, French et al. (16) succeeded in cultivating Hantaan virus in A549 cell cultures.

For 2 years, we tried to isolate Hantaan virus from blood of HFRS patients in A-549 cells but all efforts were in vain.

In 1982, McCormick et al. (30) reported that Hantaan virus produced plaques on Vero E-6 cells cultures and started to use these cells for production of large yield of Hantaan virus for EM and biochemical studies.

Then in 1983, we started to isolate Hantaan virus from blood specimens of HFRS patient directly in Vero E-6 cell cultures and finally succeeded in isolating 3 strains of Hantaan viruses from blood and serum of acute stage HFRS patients. All of the blood and serum specimens of the patients were inoculated in E-6 cell cultures as soon as the specimens were brought into laboratory. It is our knowledge that freezing and thawing of Hantaan virus in serum greatly affect the infectivity of the virus and reduces the infectivity titer by 10^{-1}, at least.

However, it seems to us that Hantaan virus in infected pulmonary tissues of rodents is not affected as much as the virus in the blood or serum to freezing and thawing.

Our preliminary experiments suggest that blood of HFRS patient is a better specimen for Hantaan virus isolation than serum. It is surprising that Hantaan virus can be isolated from bloods of patients that contained a relatively high titers of IF antibodies to the virus. Whether this suggests an excess amount of antigen relative to antibody or a dissociation of antigen-antibody complex or that IF antibody fails to neutralize the virus, it is unknown. Usually, neutralizing antibodies to Hantaan virus appear in the bloods of HFRS patients with IF antibodies during course of the illness. We are in the process of determining the presence and the amount of neutralizing antibody in blood specimens which contain both Hantaan virus, as evidenced by successful propagation in cell culture, and immunofluorescent antibodies.

Three strains of Hantaan virus isolated from blood and serum of HFRS patients were demonstrable by IF antibody technique in the cytoplasm of E-6 cells after 2nd passages in the E-6 cell cultures. Our attempts to isolate Hantaan virus from 16 urine specimens of HFRS patients in *Apodemus* mice have failed and we could not confirm the results of human experiments done by Russian scientists in 1940's (32). Further study on this question may be needed for the safety of medical personnels who are engaged in patient-care, although no man-to-man infection of HFRS has been reported yet.

It is desirable to find a susceptible line of a cell culture that produce CPE to Hantaan virus in vitro since this will reduce

from early-phase blood specimens regardless of presence of absence of antibodies.

We have isolated 3 strains of Hantaan virus from 17 blood specimens of HFRS patients in E-6 cell cultures as shown in Table 2, and all of 3 virus isolates were negative against reopolyvalent antiserum. ROK83-61 virus was isolated from heparinized whole blood but not from serum of same patient and ROK83-109 virus was isolated both from blood and serum specimens. USA84-2 virus was isolated from serum specimen but blood specimen was not available from the same patient. It is possible to isolate Hantaan virus from blood and serum specimen of HFRS patients but it seems to us that blood specimen gives better results than serum specimen.

Identification of Hantaan virus

Paired sera from HFRS patient 78/63 were used for identification of human strains of virus. These sera had titers of antibody to the 76/118 strain of virus of 1:128 and 1:16,384, respectively and did not react with reoviral antigen at a 1:20 dilution. The ROK77/137, ROK77/335 and ROK79/202 strains isolated from blood of patients that were passaged in *Apodemus* mice were compared with the prototype 76/118 virus by serum neutralization tests. Neutralization was calculated on the basis of the log of the index as shown in Table 3. The ROK83/61 and ROK83/109 strains isolated from blood of patients in E-6 cells produced clear round shape plaques on E-6 cells and plaque reduction neutralization tests of these viruses are in progress. The antigen preparations of E-6 cells infected with human strains of Hantaan virus did not react with reopolyvalent antiserum by IF antibody technique.

Attempts to isolate Hantaan virus from urine of HFRS patient

As shown in Table 4, it was unsuccessful to isolate the virus from urine specimens of early phase HFRS patients by inoculation into *Apodemus* mice although all of the urine specimens contained large masses of fibrin clots.

DISCUSSION

In the early 1940's, Japanese and Russian investigators had reported that inoculation of blood and urine from HFRS patients into monkeys (5) and human beings (32) resulted in the development of HFRS in the subjects. They also described that a filtrate of serum and urine also provoked the disease and thereby strongly suspected the infectious agent to be a virus. Many attempts have been made to isolate the etiologic agent of HFRS and clinically similar diseases for half of a century.

In 1976, Lee et al. demonstrated the presence of a specific causative antigen of HFRS in the lungs of striped field mice by reacting with the convalescent sera of HFRS patients (14). From these findings, Lee et al. confirmed that the etiologic agent of HFRS in Korea originates from its natural reservoir,

Table 4.
Attempts to isolate Hantaan virus from urine of HFRS patient in Apodemus agrarius jejuensis.

No.	Code no. of patient	Days of urine after onset	No. of Apodemus infected/no. tested
1	77/46	3	0/3
2	77/48	5	0/3
3	77/52	4	0/3
4	78/275	4	0/3
5	78/276	5	0/3
6	78/277	4	0/3
7	78/281	4	0/3
8	78/282	5	0/3
9	78/284	5	0/3
10	78/285	4	0/3
11	78/286	4	0/3
12	79/5	4	0/3
13	80/444	9	0/4
14	80/445	4	0/4
15	81/11	8	0/4
16	81/12	8	0/4

Animals were autopsied at 20 days after inoculation of 0.5 ml of urine into an Apodemus mouse intramuscularly.

Table 3.
Identity of strains of Hantaan virus isolated from HFRS patients.

Virus		Reciprocal titer in indicated serum			
		Patient 78/63 by immunofluorescence		LNI[1]	
		Acute (day 5)	Convalescent (day 10)	Human (81/73)	Rabbit (76/118)
76/118	Apodemus (passage 12)	128	16,384	5.0	4.2
ROK77/137	Patient's blood, (passaged twice in Apodemus)	64	8,192	4.6	3.8
ROK77/335	Patient's blood, (passaged twice in Apodemus)	64	16,384	4.4	4.1
ROK79/202	Patient's blood (passaged twice in Apodemus)	32	8,192	4.2	3.8
ROK83/61	Patient's blood (passaged 4 times in E6 cells)	128	8,192	Plaque reduction neutralization test is in progress.	
ROK83/109	Patient's blood (passaged 4 times in E6 cells)	128	8,192	Plaque reduction neutralization test is in progress.	

[1] LNI = log of the neutralizing index with indicated serum vs. homologous normal serum.

Table 2.
Isolation of Hantaan virus from serum and blood specimen of HFRS patient by inoculation into Vero E-6 cells.

No.	Code of patient	Sex/Age	Days of blood or serum after onset of fever		IF antibody titer to Hantaan virus	Hantaan virus isolation in Vero E6 at passage level	
1	ROK83-57	M/22	d-4	blood	128	-	P4
2	ROK83-59	M/22	d-3	serum	1,024	-	P4
			d-3	blood		-	P4
3	ROK83-60	M/26	d-6	serum	1,024	-	P4
			d-6	blood		-	P4
4	ROK83-61	M/21	d-5	serum	1,024	-	P4
			d-5	blood		+	P3(d-12)
5	ROK83-66	M/22	d-3	serum	128	-	P4
			d-3	blood		-	P4
6	ROK83-72	M/22	d-4	serum	-	-	P4
			d-4	blood		-	P4
7	ROK83-80	M/25	d-6	serum	2,048	-	P4
8	ROK83-81	M/20	d-6	serum	2,048	-	P4
9	ROK83-86	M/21	d-4	serum	-	-	P4
			d-4	blood		-	P4
10	ROK83-87	M/21	d-4	serum	1,024	-	P4
			d-4	blood		-	P4
11	ROK83-88	M/21	d-2	serum	-	-	P4
			d-2	blood		-	P4
12	ROK83-109	M/22	d-4	serum	128	+	P2(d-18)
			d-4	blood		+	P2(d-18)
13	ROK83-133	M/24	d-3	serum	1,024	-	P4
			d-3	blood		-	P4
14	ROK83-143	M/21	d-3	serum	2,048	-	P4
			d-3	blood		-	P4
15	ROK83-149	M/21	d-3	serum	512	-	P4
			d-3	blood		-	P4
16	ROK83-151	M/31	d-7	serum	512	-	P4
			d-7	blood		-	P4
17	USA84-2	M/25	d-5	serum	1,024	+	P3(d-11)

$$\frac{\text{Total no. of virus isolated}}{\text{Total no. of patient specimen inoculated}} = \frac{3}{17}$$

79-242	M/22	Febrile	3	1,024	0/4
79-246	M/23	"	3	1,024	3/4
79-263	M/37	"	4	128	0/4
79-304	M/22	"	2	<16	0/4
79-330	M/23	Oliguric	5	512	0/4
79-331	M/22	Febrile	3	256	0/4
79-332	M/20	Oliguric	7	256	0/4
79-390	M/22	Diuretic	10	256	0/4
79-460	M/22	Febrile	2	1,024	0/4
80-444	M/28	Oliguric	9	<16	1/4
80-445	M/21	"	4	256	0/4
81-11	M/22	"	8	64	0/4
81-12	M/21	"	8	<16	0/4

1/: Repeated with original blood specimens that were kept in -60° C for 10-16 months and confirmed the results.

$$\frac{\text{Total no. of virus isolated}}{\text{Total no. of blood inoculated}} = \frac{19}{105}$$

78-275	M/22	Hypotensive	4	<16	0/6
78-276	M/22	Diuretic	5	512	0/6
78-277	M/23	Febrile	4	<16	0/4
78-281	M/22	Oliguric	4	128	0/6
78-282	M/22	Febrile	5	512	0/6
78-284	M/22	"	5	128	0/3
78-285	M/21	Diuretic	4	1,024	0/3
78-286	M/23	"	4	512	0/5
78-296	M/31	Febrile	4	1,024	0/3
78-302	M/22	Diuretic	3	512	0/3
78-318	M/21	Hypotensive	6	128	3/6
78-319	M/22	Diuretic	6	128	0/3
78-320	M/23	"	4	256	0/3
78-321	M/22	"	6	128	0/3
78-346	M/23	"	6	<16	0/5
78-348	M/28	Hypotensive	4	256	2/6
78-349	M/23	Oliguric	5	1,024	0/3
78-350	M/22	"	5	256	0/3
78-371	M/22	"	6	256	0/2
78-372	M/21	Febrile	4	64	0/3
78-387	M/22	"	3	128	0/5
78-388	M/23	"	4	1,024	0/5
78-389	M/21	"	3	1,024	0/5
79-5	M/21	"	4	2,048	0/3
79-39	M/18	"	2	<16	0/6
79-69	M/24	"	3	<16	0/4
79-72	M/23	"	2	512	4/4
79-76	M/23	"	4	<16	0/3
79-78	M/23	"	1	512	1/3
79-83	M/22	"	4	2,048	1/3
79-85	M/22	"	3	<16	0/3
79-89	M/23	"	3	1,024	2/3
79-90	M/22	"	2	2,048	3/4
79-93	M/23	"	4	<16	0/4
79-103	M/24	Oliguric	5	2,048	0/4
79-104	M/22	"	5	2,048	0/4
79-129	M/22	"	5	1,024	0/4
79-130	M/22	"	5	1,024	2/4
79-131	M/22	Febrile	2	512	0/4
79-144	M/21	Diuretic	9	1,024	0/4
79-146	M/23	Febrile	3	64	0/4
79-199	M/22	"	4	64	3/8
79-200	M/22	Oliguric	8	64	0/4
79-202	M/23	Febrile	1	256	5/8
79-203	M/22	"	1	256	0/4
79-204	M/23	"	1	256	0/4
79-236	M/22	"	3	1,024	0/4
79-237	M/22	"	3	1.024	2/4
79-238	M/22	Oliguric	4	512	0/4

continued

Table 1.
Isolation of Hantaan virus from blood of HFRS patient by inoculation into <u>Apodemus agrarius</u>

Code of patient	Sex Age in yr.	Phase of illness	Days of blood after onset of fever	IF antibody titer to Hantaan virus	No. positive Apodemus/ no. inoculated Apodemus
76-109	M/21	Febrile	4	<16	4/8
76-242	M/21	"	2	<16	0/5
76-243	M/22	Oliguric	3	<16	2/7
76-253	M/21	Febrile	2	<16	0/3
76-259	M/21	"	3	<16	0/2
76-260	M/22	Oliguric	5	<16	0/5
76-267	M/22	Febrile	3	<16	0/4
76-270	M/23	"	5	<16	0/5
76-274	M/21	"	3	<16	0/4
76-288	M/22	"	2	<16	0/5
76-387	M/21	Oliguric	6	<16	0/5
77-137	M/23	"	4	512	9/10
77-184	M/21	"	6	4,096	0/3
77-216	M/22	Febrile	2	<16	0/3
77-219	M/22	Diuretic	3	128	0/3
77-231	M/22	Oliguric	5	512	0/3
77-232	M/21	Diuretic	6	2,048	0/3
77-256	M/23	Febrile	3	32	0/3
77-257	M/22	Diuretic	4	4,096	0/3
77-258	M/23	"	6	4,096	0/3
77-259	M/21	Febrile	4	512	5/7
77-299	M/26	Oliguric	4	4,096	0/3
77-300	M/22	"	5	4,096	0/3
77-308	M/23	Diuretic	4	512	0/2
77-309	M/21	Febrile	3	512	0/3
77-332	M/21	"	3	512	0/3
77-333	M/22	"	3	<16	2/6
77-335	M/23	"	3	2,048	5/6
77-350	M/22	Diuretic	5	4,096	0/3
77-351	M/22	Febrile	3	2,048	0/3
78-105	M/21	"	3	1,024	0/4
78-151	M/21	Oliguric	4	1,024	0/3
78-182	M/22	Febrile	5	256	0/5
78-189	M/22	Oliguric	4	512	0/6
78-200	M/22	Diuretic	4	1,024	0/6
78-228	M/22	Febrile	5	512	0/6
78-229	M/23	"	4	512	0/6
78-230	M/22	Diuretic	4	512	0/6
78-231	M/21	Oliguric	5	1,024	0/7
78-232	M/23	"	6	512	0/6
78-233	M/23	"	4	1,024	0/6
78-267	M/21	Febrile	3	<16	0/3
78-273	M/22	Diuretic	4	<16	0/6

continued

test in Apodemus mice (15).

From 1983, 0.1 ml of heparinized blood and 0.1 ml of serum from HFRS patient were immediately inoculated into a tube of E-6 cells as soon as the blood was brought into the Laboratory. From these cells, the supernatant and some cells were passaged into new E-6 cell culture tubes 4 times at 12-18 days intervals, depending on the condition of the cells.

At the end of each passage, the cells were examined for the presence of Hantaan virus antigen by IF antibody technique.

Fluorescent antibody technique (IAT)

For virus demonstration, the IF antibody test against Hantaan virus in pulmonary tissues of Apodemus mice and in E-6 cells was carried out as reported previously (15,16).

Neutralization test

Method of neutralization test in Apodemus mice with Hantaan viruses that were isolated from patient's blood followed by inoculation into Apodemus mice was done as described previously (15). Plaque reduction neutralization test of Hantaan virus of Dalrymple's method was also carried out in E-6 cell cultures for the identification of the virus strains that were originally isolated from patient's blood followed by multiple passages in E-6 cell cultures.

Preparation of immune sera

Normal New Zealand white rabbits were inoculated intramuscularly with 1,000 ID_{50} of Hantaan virus 76/118. On the 21st day, the rabbits were totally bled by cardiac puncture and the sera was stored at -60°C. The -log neutralization index of the hyperimmune serum was 4.5 and the titer of IF antibody to Hantaan virus was 1:4,096. Antisera of isolates from Apodemus lungs and E-6 cells were made in Wistar rats by inoculation of the virus intramuscularly.

RESULTS

Hantaan virus isolation from blood of KHF patient

As shown in table 1, 19 out of 105 blood specimens from HFRS patients yielded viral isolates when inoculated in Apodemus mice. It was previously reported that specific IF antibodies begin to appear in the blood of HFRS patients soon after the onset of illness. This observation prompted us to isolate the virus from the acute phase serum prior to the appearance of IF antibodies, resulting in isolation of virus from 4 out of 26 specimens (15.4%).

Since 1977, blood specimens were taken from HFRS patients without considering the presence or absence of antibodies. These specimens that contained IF antibodies were inoculated into Apodemus mice which yielded 15 viral isolated out of 79 specimens (19.0%). Since all blood samples from which virus isolation was possible were those collected within 6 days after onset of illness, we can conclude that virus can be isolated

INTRODUCTION

Hemorrhagic fever with renal syndrome (HFRS) is endemic in Korea and patients are known to appear in any part of South Korea (1). The reservoirs of Hantaan virus, the etiologic agent of HFRS, in Korea are not only field Apodemus mice (2,3) but also urban rats (4) and colonized laboratory rats (5,6). Lee et al. (4) reported that the urban rats captured in Seoul and four nearby Korean cities had antibodies to Hatnaan virus and that many strains of Hantaan virus were isolated from these house rats.

In the hyperendemic rural areas of HFRS, the prevalence rate of IF antibodies for Hantaan virus is about 4% among human population whereas in the less endemic area like Seoul, the prevalence rate is only 1% (1).

Currently, there are about 40,000 U.S. soldiers stationed in Korea and several HFRS cases with high fatality rate have occurred among them annually. Furthermore, the soldiers come from U.S. where HFRS is not known to exist and their stay in Korea is only for 1-2 years. Because of military importance of this severe disease and the introduction of a virgin population to this endemic area, it would be worth and also possible to find out a) the inapparent infection rate of Hantaan virus among U.S. soldiers stationed in Korea and b) the infection rate of Hantaan virus in wild urban rats caught at the U.S. Army Installations in Seoul.

Seroepidemiologic survey of U.S. soldiers and of wild rodents captured at U.S. Army Installations in Seoul and DMZ have started in collaboration with the 5th Preventive Medicine Unit, the U.S. Army Hospital, the 8th U.S. Army in Seoul and The Institute for Viral Diseases, Korea University.

MATERIALS AND METHODS

One to two ml of serum from a healthy soldier collected at the U.S. Army Hospital and wild urban rats caught by means of baited live traps at the Army Installations were brought to our Institute immediately. Processing of these rodents, antibody examination of sera, and detection of Hantaan virus from lungs of rats were carried out by the methods described previously (3). 1986 sera from healthy soldiers in 1982 and 1983, and 141 urban rats caught at the U.S. Army Installations in Seoul and 2 wild rats caught in the DMZ area in 1983 were examined for this study.

RESULTS

Number of HFRS patient and occurrence of IF antibodies to Hantaan virus among U.S. Army stationed in Korea.

Table 5 shows no. of HFRS patient hospitalized at the U.S. Army Hospital who were serologically confirmed by our Institute

Table 5.
Number of HFRS patients among U.S. soldiers stationed in Korea serologically confirmed at the Institute for Viral Diseases, Korea University.

Year	No. of hospitalized patient	No. of death
1980	1	0
1981	1	1
1982	2	1
1983	3	1
1984 (July)	1	0

Table 6.
Occurrence of immunofluorescent antibodies to Hantaan virus among U.S. soldiers stationed in Korea.

	Year	
	1982	1983
$\frac{\text{No. of antibody positive}}{\text{No. of serum tested}}$ (%)	15/991(1.5%)	9/995(0.9%)

Table 7.
Immunofluorescent antibodies to Hantaan virus and pulmonary viral antigen in wild rats caught at the U.S. Army Installations in Seoul and DMZ in 1983.

Locality	Rattus norvegicus			Rattus rattus			Total	Positive (%)	
	No. tested	Antibody present	Antigen present	No. tested	Antibody present	Antigen present		Antibody	Antigen
8th US Army Installations in Seoul	89	11	2	52	3	0	141	9.9	1.4
DMZ	0			2	1	0	2	5.0	0
	89	11	2	54	4	0	143		

from 1980 to July 1984. There were only a few patient with very high fatality rate every year.

The prevalence rate of IF antibodies to Hantaan virus among about 2,000 healthy U.S. soldiers is shown in Table 6 and the percentage of antibody positive is about 1.2% which is a data very similar to that of Seoul residents.

Detection of IF antibodies to Hantaan virus and of viral antigen in urban rats.

We examined 89 *R. norvegicus* and 52 *R. rattus* caught at U.S. Army Installations in center of Seoul, and 2 *R. rattus* caught in the DMZ. The details of animals tested and those positive for antibody and antigen are shown in Table 7. Evidence of infection of rats with Hantaan virus-like agent is clear and positive rat is about 10% among 144 rats tested. We found only 2 rat's lungs positive for antigen but did not try to isolate the virus since we had isolated so many strains of Hantaan-like virus from urban rats caught in other districts of Seoul in 1981.

DISCUSSION

There were only a few cases of HFRS patients hospitalized at the U.S. Army Hospital serologically confirmed at our Institute but the fatality rate was very high. This may be due to the inexperience of medical doctors with HFRS patients and due to the transportation of severe cases with renal failures from Seoul to U.S. which affects the course of this illness very badly. For the HFRS patients who have hemorrhagic tendencies, absolute rest accompanied by rapid symptomatic treatment are the most important measures to save the lives from this severe disease.

Subclinical infection rate of Hantaan virus among U.S. soldiers is almost equivalent to that of the resident of Seoul and to that of Korea soldiers (1). These facts indicate that there is no difference in susceptibility to Hantaan virus among different races and that Hantaan and related agents are widely distributed in U.S. Army Installations throughout the Korean peninsula.

Rodent control should be continued in the aras of U.S. Army Installations in Korea because it has been proven that urban rats infected with Hantaan or related agents exist and that the infection rate of rats in those areas is same as that of other districts of Seoul. It should be mentioned that 50 - 100 severe cases of HFRS patients among residents in the metropolitan area of Seoul were hospitalized every year since 1980 (7,8).

CONCLUSION

Fatality rate of HFRS patient in the U.S. Army Hospital in Seoul is very high. The prevalence rate of IF antibodies to Hantaan virus among 1,986 U.S. soldiers stationed in Korea was

1.2% which is a data a very similar to that of Seoul residents and Korean soldiers. Urban rats captured at the U.S. Army Installations were found to have IF antibodies to Hantaan virus, the etiologic agent of HFRS. Serum antibodies were detected in 12.3% of 141 R. norvegicus and in 7.4% of 54 R. rattus. Hantaan viral antigen was found in lung tissues of 2 rats.

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C. DISTRIBUTION OF IMMUNOFLUORESCENT ANTIBODIES TO HANTAAN VIRUS IN DOMESTIC ANIMALS OF KOREA, JAPAN AND HONG KONG.

INTRODUCTION

It was proved recently that urban rats and laboratory rats, as well as field mice are the reservoirs of Hantaan and related viruses(1,2,3,4,5,6,7), the etiologic agent of Hemorrhagic fever with renal syndrome (HFRS), and that HFRS cases occurred in urban areas and some animal's rooms. However, any seroepidemiologic examinations for Hantaan viral infection in domestic animals that are in close contact with urban or laboratory rats have never been done because animals have not shown any signs of disease due to the infection with Hantaan virus (8).

We report here for the first time the presence of immunofluorescent antibodies (IF) to Hantaan virus in the sera of domestic animals in Korea, Japan and HongKong.

MATERIALS AND METHODS

Sera of cattle, rabbits, chickens, dogs, porcine, goose and ducks:

Cattle's blood was obtained from cattle slaughterhouse; rabbits' from 2 rabbit-breeding houses, Korea and from a breeding Co., Japan; chicks' from 5 chick markets in Seoul; dogs' from a dog market in Jungang Market place in Seoul; porcines' from 7 pig slaughterhouses in the suburbs of Seoul. Each animal's serum was separated from its blood. In addition to above animal sera, 50 cattle sera, 50 porcine sera, 49 chicken sera, 50 goose sera and 50 duck sera were obtained from Dr. K.F. Shortridge in Hong Kong.

Hantaan viral antigen:

A549 cells infected with the 76-118 strain of Hantaan virus was used as an antigen (9). The indirect immunofluorescent antibody method was used for detection of IF antibodies to Hantaan virus, and the details of this method were described as before (2).

RESULTS

Distribution of IF antibodies to Hatnaan virus in domestic animals of Korea, Japan and Hong Kong.

The sera of 110 heads of cattle slaughtered in many cattle slaughterhouses were examined by IF antibody technique and they were negative for antibody to Hantaan virus. The examinations of 50 dogs bred in the suburbs of Seoul,,and of 50 goose and 50 ducks slaughtered in Hong Kong also showed the same results. However, 4 of 35 rabbits bred in Seoul, and 3 of 35 rabbits in Dongducheon where many HFRS cases have often occurred were shown to have antibodies to Hantaan virus. Similar results were also obtained in the sera of rabbits from Japan. As shown in Table 8, 22 rabbits were antibody positive for Hantaan virus and some positive sera contained very high antibody titers, 1:512 - 1:1,024. One of 123 chickens bred in the suburbs of

Table 8.
Distribution of immunofluorescent antibodies to Hantaan virus in the sera from domestic animals in Korea, Japan and Hong Kong, 1981-1983.

Animal	Location		No. positive/ no. tested		IF antibody titers of positive serum
Rabbit	Seoul	Breeding Co.	4/35	Total (29/792) [3.5%]	Antibody titers ranged 16 - 1,024; mean value:64.
	Dongduchun	Farmer's house	3/35		
	Japan	Breeding Co.	22/722		
Chicken	Yongsan	Market	0/18	Total (1/123)	AE-Chick-82-27(1:256)
	Sugkwan	"	0/12		
	Chungryangri	"	1/27		
	Sooyoo	"	0/17		
	Hong Kong		0/49		
Porcine	Kwangju	Abattoir	0/24	Total (1/104)	P-1423 (1:64)
	Kangwha	"	0/13		
	Pochun	"	0/17		
	Hong Kong		1/50		
Cattle	Ansung	Abattoir	0/22	Total (0/100)	
	Paju	"	0/16		
	Kwangju	"	0/12		
	Hong Kong		0/50		
Dog	Chungang Market		0/50		
Goose	Hong Kong		0/50		
Duck	Hong Kong		0/50		

Seoul had very high titer of IF antibodies to Hatnaan virus and one of 104 porcine bred in Hong Kong also had IF antibodies.

DISCUSSION

In 1978, some normal rabbits were bought from several breeding Co. in Seoul to immunize with Hantaan virus to make antisera, but it was found that these rabbits were already antibody positive with IFA test. Since that time, we have been examining all sera from rabbits to be used in the experiments for antibody to Hantaan virus. One of the reservoirs of Hantaan virus is wild house rats which are cosmopolitan animals that live very closely with domestic animals.

As we demonstrated in this paper, about 5 % of commercial rabbits are antibody positive for Hantaan virus which suggests that they were already infected either in the Animal Farms or in the private rabbit breeding houses. However, the role of rabbits in the natural cycle of Hantaan virusis not known. It is our recommendation that all rabbits must be checked for Hantaan virus infection prior to use in Hantaan virus experiments. Also, the significance of one antibody positive chicken out of 123 and one positive porcine from Hong Kong for Hantaan virus remains to be studied. Further studies on neutralizing antibodies from IF antibody positive domestic animals are needed to better understand the ecology of Hantaan and related virus in nature.

CONCLUSION

Commercial rabbits bought from breeding companies in Korea and Japan were found to have IF antibodies to Hantaan virus. Serum antibodies were demonstrated in 3.5% of 792 New Zealand white rabbits. One out of 123 chicken sera and one of 104 porcine sera were IF antibody positive for Hantaan virus. However, dogs, geese, ducks and cattles were negative for Hantaan virus. The significance of positive sera for Hantaan virus from domestic animals in the endemic areas of HFRS remains to be studied.

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